



Effects of pulsed electric fields (PEFs) on fermentation quality characteristics and microbial community of kimchi

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ARTICLE INFO

Keywords:

Kimchi
Pulsed electric fields
Microbial community
Fermentation
Multiple comparison test

ABSTRACT

Kimchi, a lactic acid bacteria (LAB)-fermented food, is consumed globally at approximately 1.5 million tons annually. This study evaluated, for the first time, the effect of pulsed electric field (PEF) pretreatment on the microbial community of kimchi. Chinese cabbage was pretreated at 1.5 kV/cm, brined with varying NaCl concentrations (5, 10, or 15 g/L), and fermented at 4 °C for 25 days. PEF pretreatment effectively maintained cellular integrity and volume. The dominant microbial taxa differed based on pretreatment, with *Weissella* prevailing in PEF-treated groups and *Lactobacillus* in untreated samples. PEF pretreatment inhibited *Lactobacillus* growth, the primary lactic acid producer, resulting in distinct sensory profiles compared with untreated samples. The PEF-treated group exhibited a slower LAB growth rate, with fermentation delayed by approximately 5–7 days, compared with the untreated group. These findings suggest that PEF pretreatment can effectively control the fermentation and microbial composition of kimchi, extending the shelf life.

1. Introduction

Lactic acid bacteria (LAB) are gram-positive, non-sporulating microorganisms that produce functional compounds as secondary metabolites during fermentation (Khalid, 2011). These compounds determine the fermentation quality, texture, and sensory properties (Lee et al., 2017). Kimchi, an LAB-fermented food, is a brined and fermented vegetable consumed at approximately 1.5 million tons annually and comprises various bacteria, yeast, and viruses (Baek et al., 2023). The major LAB species identified in kimchi include *Lactobacillus*, *Leuconostoc*, *Weissella*, and *Lactococcus*, which contribute to its unique carbonated and acidic flavor during fermentation (Zabat et al., 2018). However, the composition of these microbial communities can vary depending on the fermentation period, NaCl distribution during brining, ingredients, and storage conditions (Lee et al., 2017).

Excessive and rapid LAB growth can impart a bitter taste, sourness, and off-odor to kimchi and deteriorate its texture due to over-

acidification (Lee et al., 2021). Moreover, fermentation failure can accelerate excessive microbial growth, leading to food spoilage and the production of undesirable, tasteless, and unpalatable kimchi (Song et al., 2020). To address these issues, strategies such as high hydrostatic pressure (Lee et al., 2019), supercooled storage (Lee et al., 2021), irradiation (Song et al., 2009), and addition of antimicrobial agents (Kim et al., 2012) have been applied during the brining and fermentation of kimchi. However, previous research on brining technologies has not fully explored the microbial community of kimchi in relation to the quality of the fermentation products (storage period and sensory properties). Additionally, these technologies may not be suitable for commercial kimchi production because of poor consumer acceptance, degradation of nutritional compounds, altered texture and sensory properties, and high energy requirements.

Pulsed electric field (PEFs), a non-thermal food preservation technology, uses short high-voltage pulses to create reversible and irreversible pores in cell membranes through electrolytic reactions (Niu

Abbreviations: TA, Titratable acidity; TLAB, Total lactic acid bacteria; PEF, Pulsed electric fields.

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<https://doi.org/10.1016/j.lwt.2025.118942>

Received 13 August 2025; Received in revised form 15 October 2025; Accepted 18 December 2025

Available online 17 January 2026

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et al., 2020). These pores increase cell membrane permeability and accelerate mass transfer during various food-processing operations such as brining, drying, rehydration, and freezing (Nowosad et al., 2021). Specifically, applying PEF during the brining process can drastically increase the NaCl absorption rate and ensure homogeneous NaCl distribution, thereby inhibiting early LAB growth without deteriorating the cellular structure (Kim et al., 2023). However, the application of excessively strong PEF can accelerate the destruction of not only the cell membrane but also the cell wall, thereby increasing the amount of nutrients available to microorganisms and promoting microbial growth, so the establishment of optimal conditions is necessary (Williams et al., 2019). Several studies have applied PEF to enhance mass transfer during the brining of sea bass (Crobotova et al., 2021), Chinese cabbage (Kim et al., 2023), and lotus roots (Li et al., 2021). However, few studies have investigated the secondary effects of PEF after brining, which is particularly important from an industrial perspective. Although PEF has demonstrated strong potential in accelerating mass transfer and maintaining product quality during brining, its industrial adoption remains limited due to concerns regarding energy efficiency and process cost (Toepfl et al., 2014). From an industrial standpoint, the ability to shorten brining time and reduce NaCl concentration without compromising quality would provide significant economic and environmental benefits (Vega-Mercado et al., 1997). Shorter brining durations can improve production throughput and reduce storage and energy requirements, while lower salt concentrations can minimize raw material usage and decrease effluent salinity, thereby reducing wastewater treatment costs and environmental impact (Vega-Mercado et al., 1997; Toepfl et al., 2014; Kim et al., 2023). Therefore, understanding how PEF influences fermentation kinetics and microbial dynamics after brining is essential to assess its feasibility as a sustainable and cost-effective technology for large-scale kimchi production.

We previously explored the mechanism of accelerated brining in Chinese cabbage pretreated with PEF and observed homogeneous NaCl distribution and maintenance of cellular structure during the brining and fermentation processes (Kim et al., 2023, 2024). Building on these studies, we aimed to examine the effects of combining PEF with varying concentrations of NaCl brining solutions on kimchi fermentation. Chinese cabbage was pretreated with PEF (1.5 kV/cm), a strength determined by prior research, and then brined with different NaCl solutions (5 g/L, 10 g/L, and 15 g/L) until reaching a salinity of 1.5 %. Subsequently, the morphology, general fermentation quality characteristics, and microbial communities during fermentation were investigated. The initial growth rate of LAB affects sensory properties because of the varying composition of LAB in kimchi. Thus, we conducted a sensory evaluation of kimchi at the optimal general fermentation period based on the no PEF-S10 group (day 15, pH 4.0–4.5). To the best of our knowledge, this is the first investigation examining how PEF application during kimchi brining influences fermentation quality and microbial composition. Focusing on the interplay between PEF-assisted brining and fermentation dynamics, the present work integrates kinetic, microbial, and sensory analyses to provide new insights into the potential of PEF as a sustainable strategy for enhancing brining efficiency and reducing salt usage in industrial kimchi production.

2. Materials and methods

2.1. Kimchi preparation

Chinese cabbage was harvested in December and purchased from a local farm in Gangwon-do, Korea. Seasoning ingredients used for kimchi preparation, including red pepper powder, garlic, and ginger, were purchased from a local market in Jeollanam-do, Korea. They were stored in a freezer and thawed before use in the experiment. Chinese cabbage was cut into fixed sizes (30 × 30 × 7 mm) and prepared for brining with 10 g/L NaCl solution for 3 h to reach the desired 1.5 % salinity (No PEF-S10, untreated group). After cutting, the Chinese cabbage underwent

PEF treatment using a 5-kW pulse generator (HVP-5, DIL, Quakenbrück, Germany) under fixed conditions (20 µs pulse width, 50 Hz pulse frequency, and 500 pulses, at a temperature of 25 °C) in a batch treatment chamber with parallel gap electrodes (80 mm). Approximately 50 g of the sample was mixed with 200 mL of tap water and placed in the chamber. The strength of the PEF was fixed at 1.5 kV/cm, as per our previous research (Kim et al., 2023). After PEF pretreatment, the sample was brined with 5 g/L (1 h), 10 g/L (30 min), and 15 g/L (15 min) NaCl solutions until 1.5 % salinity was reached to investigate the effects of the NaCl concentration in the brining solution (only for the PEF treatment group). Reflecting the food industry level, which generally uses a 10 g/L NaCl solution for the brining process, no PEF-S10 was brined with 10 g/L. To compare the differences between the NaCl concentrations in the brining, the NaCl concentration of the brining solution was divided into 5, 10, and 15 g/L, which influenced brining time. The samples were designated as follows: no PEF-S10 (untreated group brined with 10 g/L NaCl solution), PEF-S05 (PEF treatment group brined with 5 g/L NaCl solution), PEF-S10 (PEF treatment group brined with 10 g/L NaCl solution), and PEF-S15 (PEF treatment group brined with 15 g/L NaCl solution). After the brining process, the brined Chinese cabbage was mixed with kimchi seasoning in a 3:1 ratio and stored at 4 °C.

2.2. Morphological structure

The cellular structure of the brined samples was observed under a light microscope (BX51; Olympus, Tokyo, Japan) to investigate the effects of PEFs during the brining process. Samples were prepared by fixation, dehydration, sectioning with an ultramicrotome (Ultracut R; Leica Co., Vienna, Austria), and staining following the periodic acid-Schiff method described by (Park et al., 2022).

2.3. Kinetic modeling of total lactic acid bacteria (TLAB) growth

Based on the TLAB experimental data, the Logistic and Gompertz models (Gibson et al., 1987) were used to fit the growth curve of LAB according to a previously reported method using GraphPad Prism 8.0.1. software (GraphPad Software, Boston, MA, USA) (Juneja et al., 2009):

$$y(t) = y_0 + (y_{max} - y_0) \exp(-\exp(-B(t - M))) \quad (1)$$

$$y(t) = y_0 + \frac{(y_{max} - y_0)}{1 + \exp(-B(t - M))} \quad (2)$$

where $y(t)$ is the microbial count at time t , y_0 is the microbial count at the initial time (d), M is the time at which the absolute growth rate reaches its maximum, and B is the maximum relative growth rate at t . The goodness of fit of the primary models was evaluated using the regression coefficient (R^2), adjusted coefficient of determination (R^2_{adj}), root mean square error (RMSE), and sum of squares (SumSq).

2.4. General fermentation quality characteristics

The pH, titratable acidity (TA), salinity, and TLAB counts were determined to evaluate the general fermentation quality characteristics. Measurements were conducted according to methods described by the World Institute of Kimchi (WIKIM, Gwangju, Republic of Korea). To prepare the sample extract solution, 20 g of the sample was filtered through 100 % absorbent gauze (7.5 × 7.5 cm) using a chopping machine (CTG-14-GPKR, Huahai Plastic Products Co., Ltd, Guangdong Province, China). The pH of each sample extract (20 mL) was measured using a pH meter (TM S210; Mettler Toledo, Greifensee, Switzerland). To determine the TA, 10 mL of sample extract was mixed with 90 mL distilled water, and the pH was adjusted to 8.3 using 0.01 N NaOH; then, the quantity of 0.01 N NaOH consumed was calculated as described by (Lee et al., 2021). The salinity was measured using a salinometer (SALT-FREE2500, Ohio, USA). To enumerate the TLAB, 10 g of each

sample was homogenized and serially diluted in saline, following the method described by Lee et al. (2019) with slight modifications. Diluted sample (1 mL) was plated on lactic acid bacteria petrifilm (3M Co., St. Paul, MN, USA) and incubated at 37 °C for 24 h. After incubation, colony counts were expressed as log CFU/mL.

2.5. Sensory evaluation

To evaluate the sensory properties of kimchi, a multiple comparison test was conducted with ethical approval granted by the Institutional Review Board (IRB) of Chung-Ang University (1041078-20230117-HR-009). Written informed consent was obtained from all participants prior to their involvement in the sensory evaluation. The multiple comparison test (ISO, 2006) was performed with slight modifications. A trained panel of 16 individuals comprising graduate students and staff at Chung-Ang University participated in this experiment. To train the panel, precise definitions and descriptions of the evaluation characteristics of kimchi were provided before supplying the food samples according to the method described by Watts et al. (1989). Approximately two-thirds of the panelists (13/16) were regular consumers of kimchi (once daily), and one-third of the panelists (3/16) were occasional consumers who consumed kimchi three times weekly. Kimchi samples (pH 4.0–4.5, fermented on day 15) were prepared and labeled with random numbers. The panelists received four samples in a plastic dish (10 g) on white plates containing rice (10 g) and water (250 mL). Among the four samples (no PEF-S10 and PEF-treated samples), the multi-sample comparator first tested the contrast (no PEF-S10), and then compared with the contrast sample. The degree of difference (−4 to +4, contrast 0) was applied on a 9-point scale (slight difference compared to no PEF-S10 = 1, no difference compared to no PEF-S10 = 5, and strong difference compared to no PEF-S10 = 9). Differences in the food characteristics between the samples were comprehensively identified by considering their appearance, flavor, texture, and taste.

2.6. Library preparation and sequencing of bacterial 16S rRNA gene

To investigate the microbial community composition, sample extracts were prepared by centrifugation at 18,000×g for 10 min at 4 °C. A QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) was used to extract total genomic DNA from the sample pellets. The 16S rRNA gene was amplified using the Illumina-adapted universal primers 341F and 805R, targeting the V3–V4 region. Polymerase chain reaction (PCR) was performed with ExTaq polymerase (Takara Bio, Kusatsu, Japan), starting with an initial denaturation at 98 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The PCR products were purified and quantified using AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and a Qubit dsDNA High-Sensitivity Assay Kit (Invitrogen; Carlsbad, CA, USA). Normalized amplicons were prepared for sequencing using a Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) in combination with a Nextera XT Index Kit. Sequencing was performed on a MiSeq platform using the MiSeq Reagent Kit v3 (600 cycles) (Illumina).

2.7. Analysis of 16S rRNA gene sequence data

Sequencing reads were demultiplexed and paired-end reads joined, followed by quality filtering using the QIIME2 pipeline (version 2024.5) (Bolyen et al., 2019). Next, the DADA2 workflow was applied, which uses sequence error profiles to obtain high-quality amplicon sequence variants (ASVs) (Callahan et al., 2016). ASVs were then assigned a taxonomy using the Greengenes 2 reference database (2022.10) through the q2-greengenes plugin within QIIME2 (McDonald et al., 2024). Subsequent microbial analyses, such as beta diversity metrics (non-metric multidimensional scaling (NMDS) and Bray–Curtis distance) and linear discordant analysis of Effect Size (LefSe), were

conducted using MicrobiomeAnalyst (Lu et al., 2023). The 16S rRNA gene amplicon datasets obtained in this study were deposited at the National Center for Biotechnology Information (NCBI) under the accession numbers.

2.8. Kimchi metabolome analysis by ¹H NMR spectroscopy

Cha's method was used for metabolomic analysis, with slight modifications (Cha et al., 2018). Briefly, kimchi supernatants (300 µL) were combined with 300 µL of mix solution (60 µL of deuterium oxide containing 0.3 mM 2-[trimethylsilyl] propionic acid-d₄ sodium salt [TSP], 60 µL of 5 mM imidazole, 60 µL of 2 mM Na₃N₃, and 120 µL of 1 M potassium phosphate buffer). The nuclear magnetic resonance (NMR) spectra were obtained using a Varian 500 MHz NMR system (Varian, Palo Alto, CA, USA) equipped with a cold-flow probe. The spectra were collected using a water pre-saturation pulse sequence with 64 transients. The spectra were corrected using the Chenomx NMR Suite version 11.0 (Chenomx, Canada). The metabolites were quantified using the built-in database of Chenomx NMR Suite 11.0.

2.9. Statistical analysis

The analysis of variance (ANOVA) in IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA) was used to analyze the data and expressed as mean ± standard deviation. The Duncan's multi-range test and Tukey's post hoc test with considering statistically significant at $p < 0.05$ to determine the differences in the mean. GraphPad PRISM (version 10) was used for statistical comparison and visualization (bar plot) of the microbiome and metabolome data. The R package Pheatmap was used to draw the metabolome heatmap and MetaboAnalyst 6.0 was used to analyze the metabolite profiles and volcano plots (Pang et al., 2024).

3. Results and discussions

3.1. Morphological structure changes

Cell wall morphological structures are shown in Fig. 1. After PEF pretreatment and brining with different NaCl concentrations of brining solution, the cell structure and volume were maintained better than those in the no PEF-S10 group. At low NaCl concentrations, uniform and intact cellular structures were observed. However, despite the decrease in the brining time, the higher the NaCl concentration in the brining solution, the more drastic the damage to the cell structure of the PEF-treated group. However, regarding the overall morphology, PEF pretreatment improved cell structure and volume compared with that in the no PEF-S10 group.

In general, during the brining process osmotic pressure induces plasmolysis, which indicates the disruption of cell structure, loosening of cellular structure, and stacking of cell walls (Kim et al., 2024). The high NaCl concentration in the brining solution accelerated the material movement and NaCl penetration into the cells. Thus, a high NaCl concentration in the brining solution maximizes the osmotic impact and rapidly collapses intact cell structures (Kim et al., 1988). This process creates an environment that is useful for LAB growth because the nutritional components leak out from the inner cells and accelerate the fermentation rate owing to the formation of an anaerobic state as storage time increases (Kim et al., 2023).

Similarly, we discovered that PEF pretreatment improved the maintenance of Chinese cabbage cell structure during the brining process, with a homogeneous NaCl distribution. Furthermore, in our previous study, radishes pretreated with PEF maintained cell volume and an intact cell structure after the brining process (Kim et al., 2024). Considering these results, PEF pretreatment may improve the maintenance of cell structures in Chinese cabbage, regardless of the NaCl concentration of the brining solution, compared with no PEF-S10 group.

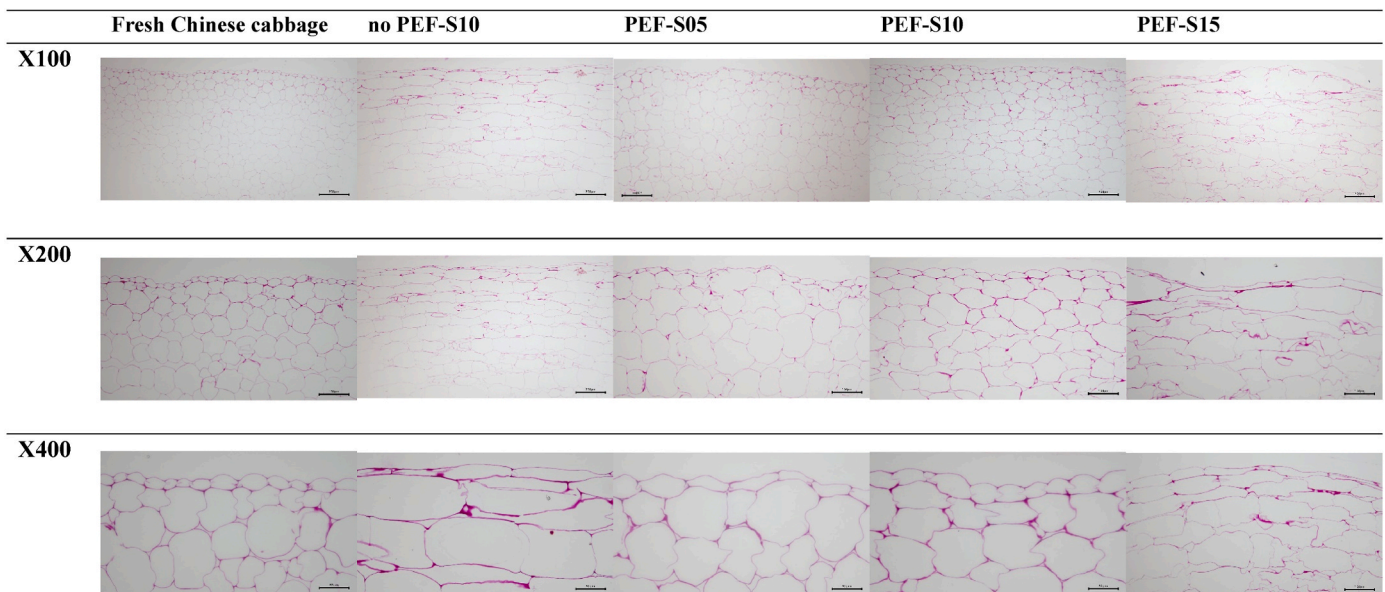


Fig. 1. Morphology of brined Chinese cabbage pretreated by Pulsed electric fields (PEF) with different concentration of NaCl. Fresh Chinese cabbage: fresh sample, no PEF S-10: No treatment of PEF then brining with 10 g/L NaCl solution, PEF-S05: sample pretreated by PEF and brining using 5 g/L NaCl solution, PEF10: sample pretreated by PEF and brining using 10 g/L NaCl solution, PEF-S15: sample pretreated by PEF and brining using 15 g/L NaCl solution.

3.2. Distinct alteration in microbial community of PEF-treated kimchi

A relative abundance analysis was performed for the 20 most dominant bacterial species detected during the fermentation process (Fig. 2). The kimchi produced in this study contained microbial species such as *Weissella*, *Lactobacillus*, and *Leuconostoc*, which is consistent with previously reported findings (Song et al., 2020). This confirmed that the observed microbial patterns were similar to those of garlic-based fermented kimchi (Song et al., 2020). The PEF-treated and untreated groups exhibited distinct shifts in microbial communities during fermentation. Notably, *Latilactobacillus* was significantly reduced in the PEF-treated groups than in the no PEF group (Fig. 2a). Within the PEF-treated groups, differences were also observed depending on the salt concentration, where a high salt content appeared to prolong the presence of the initial microbial species, contributing to delayed fermentation. The microbial differences between groups were minimal on day 1 but became increasingly evident by day 10. In the no-PEF group, fermentation proceeded rapidly, with a corresponding increase in the main microbial species. In contrast, PEF-treated groups exhibited delayed fermentation, which was prominent between days 15 and 25. To further explore these differences, LEfSe analysis was used to identify microorganisms that were significantly enriched in each group at the same salt concentration. LEfSe highlighted distinct microbial species unique to the PEF-treated and untreated groups. To validate the results of the LEfSe analysis, a comparison of the relative abundance of LAB was performed for the no PEF-S10 and PEF-S10 groups during the late fermentation period (days 15–25). The findings revealed that *Weissella* and *Leuconostoc* were dominant in PEF-treated kimchi, suggesting their critical roles in the fermentation process (Fig. 2d). Notably, although microbial evenness (uniformity of species distribution) was lower in the PEF-treated samples, microbial richness (total number of species) did not differ significantly, indicating that even less abundant microorganisms may play important functional roles within the ecosystem.

These results demonstrate that PEF treatment not only delays fermentation, but also influences the composition of LAB, resulting in unique fermentation dynamics and sensory profiles. The specific LAB composition, dominated by *Weissella* and *Leuconostoc* likely contributes to the extended “green” flavor and delayed sourness observed in PEF-

treated kimchi. *Weissella confusa* is usually detected in juice, raw milk, and sugar cane, whereas *Leuconostoc citreum* is commonly found in various fermented foods and contributes to antibacterial and antifungal activities (Lee et al., 2005). In particular, the *Weissella* genus is a dominant catalase-negative bacterium that produces lactic acid, acetic acid, and carbon dioxide, which influence the fresh taste and flavor, and have anticancer, anti-inflammatory, blood cholesterol-lowering, and antioxidant effects (Kwak et al., 2014). The *Lactobacillus* genus, which is an anaerobic and facultative anaerobic gram-positive bacterium, imparts an acidic taste to kimchi by producing various organic acids (Kwak et al., 2014). Park et al. (2014) reported that on the genus level, *Weissella* accounted for the highest portion (27.2 %), followed by *Lactobacillus* and *Leuconostoc*, using pyrosequencing at the optimal stage of kimchi fermentation. Furthermore, as NaCl concentration in the brining solution increased, *Weissella* increased and *Lactobacillus* decreased (Hong et al., 2021). *Weissella* and *Leuconostoc* generate carbon dioxide that contributes to carbonated and fresh flavors. *Lactobacillus* generates lactic acid, which increases the acidity and taste of kimchi. Overall, pretreatment with PEF inhibited the early growth of LAB, and the relatively homogeneous NaCl distribution in brined Chinese cabbage contributed to the growth of *Weissella*, which influenced the fresh flavor; thus, the green taste and flavor were enhanced for a longer time compared with those of no PEF-S10. These microbial dynamics may offer valuable insights into optimizing PEF-based kimchi production, providing enhanced quality and prolonged freshness.

3.3. Kimchi metabolite changes based on PEF pretreatment

A quantitative analysis was performed for the 30 metabolites abundantly present in kimchi, and the results are shown in Figs. 3 and 4. In the no PEF group, amino acid compounds began to increase from day 5, with representative flavor-related substances such as mannitol and lactate peaking on day 15. In contrast, the PEF-treated group showed a delayed increase in most compounds, with notable changes observed on days 15 and 25, indicating a clear delay in fermentation. Despite this delay, PEF-treated kimchi on day 25 exhibited the highest concentration of mannitol, one of the key quality factors for desirable kimchi, along with elevated levels of branched-chain amino acids such as isoleucine,

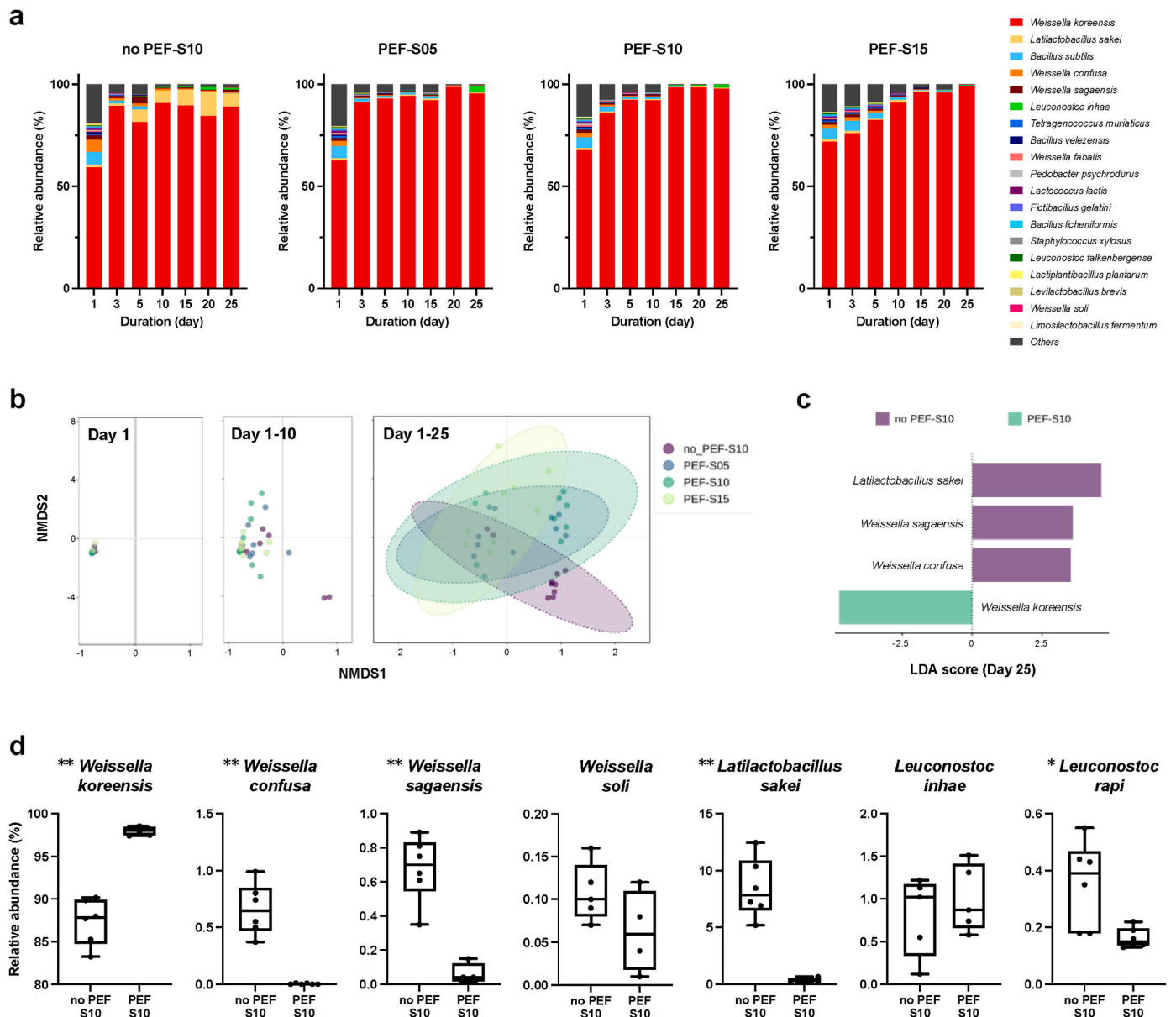


Fig. 2. Analysis of bacterial community of kimchi based on several PEF pretreatment conditions. (a) Taxa bar plot of kimchi microbiome during fermentation at the species level. (b) Non-metric multidimensional scaling (NMDS) plot for the comparison of beta diversity of kimchi microbiome between each group. (c) Linear discriminant analysis (LDA) showing specific microbiota between no PEF and PEF treatment groups with brining using 10 g/L of NaCl at 25 day. (d) Relative abundance of major kimchi lactic acid bacteria between no PEF-S10 and PEF-S10 groups at 15–25 day. no PEF-S10: No treatment of PEF then brining with 10 g/L NaCl solution, PEF-S05: sample pretreated by PEF and brining using 5 g/L NaCl solution, PEF10: sample pretreated by PEF and brining using 10 g/L NaCl solution, PEF-S15: sample pretreated by PEF and brining using 15 g/L NaCl solution. Statistical significance was determined using Mann-Whitney test ($n = 6/\text{group}$; **, $p < 0.01$; *, $p < 0.05$).

leucine, and valine. Moreover, metabolites such as formate and fumarate were significantly increased in the PEF-treated group, similar to 4-aminobutyrate (GABA), a compound of growing interest owing to its functional properties. These findings highlight the potential of PEF treatment for producing kimchi with distinct and desirable quality characteristics, which could have significant implications for industrial applications. Notably, the taste-related components of kimchi differed depending on whether the PEF treatment was applied at the same salt concentration. Mannitol levels further increased in PEF-treated kimchi, whereas ethanol and acetate levels decreased. This suggests that PEF treatment enhances umami flavors, while reducing sourness and fermented flavors. Similarly, the results of the sensory evaluation also revealed significant differences between the PEF-treated and untreated groups in terms of sourness and flavor (Fig. 5e). Distinct trends were

observed when comparing the metabolites of the no PEF-S10 and PEF-S10 groups during the fermentation period (Fig. 4). On day 5, the no PEF-S10 group produced more metabolites, indicating fast fermentation. This trend peaked on day 15, except for fumarate and formate, which showed higher levels in the untreated group. By day 25, the fermentation process in the PEF-treated group had progressed, leading to a reduction in group-specific compounds.

3.4. Kinetic TLAB modeling during fermentation

To determine TLAB growth, the critical kinetic parameters, y_0 , $y_{\max}$, B , and M , were investigated (Table 1). The logistic and Gompertz models are traditional primary models for estimating microbial growth under constant temperature (Juneja et al., 2009). Higher B values and

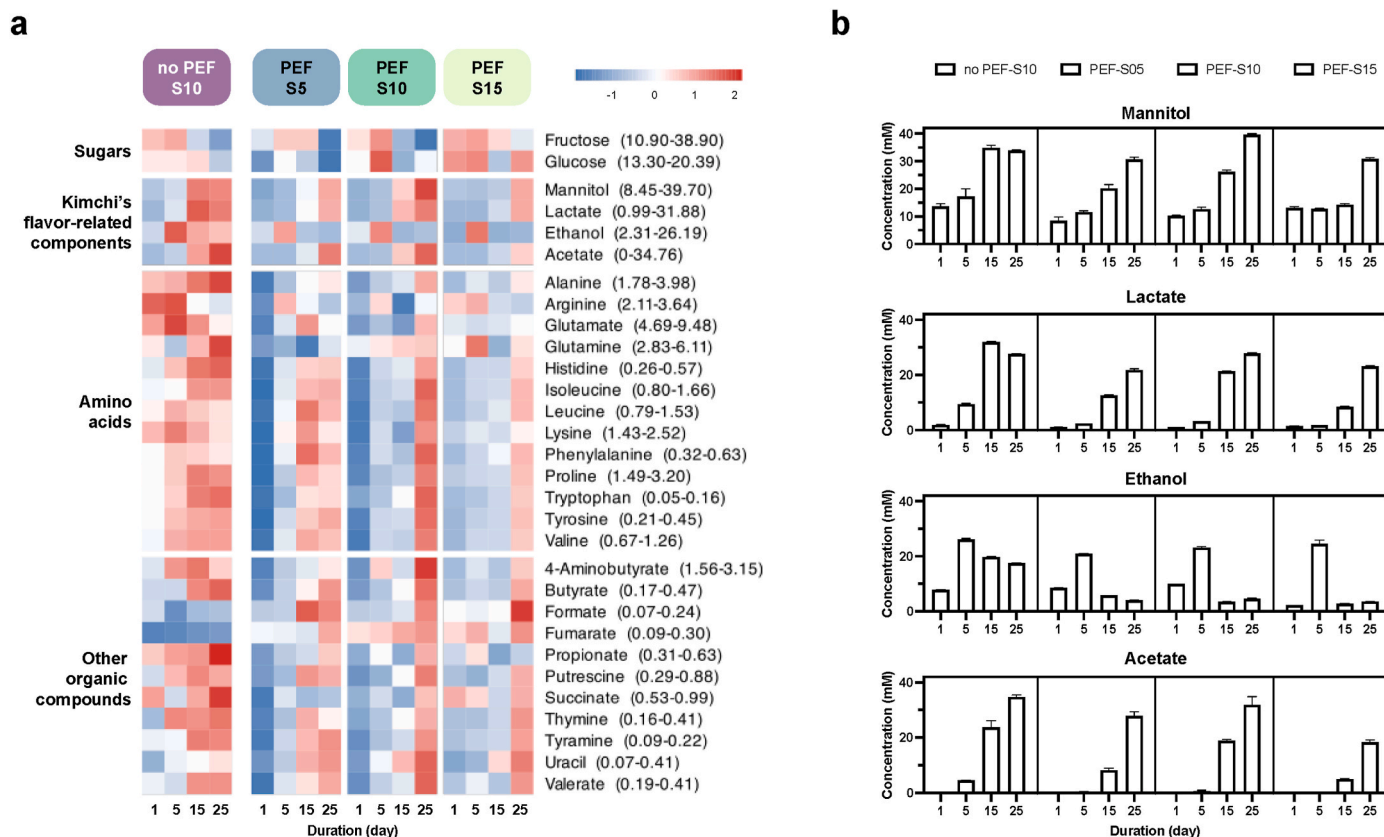


Fig. 3. Analysis of kimchi metabolites in each pretreatment group during fermentation (1, 5, 15, and 25 days). (a) Heatmap of 30 key metabolites from kimchi. The metabolites were separated into 4 category (2 sugars, 4 kimchi's flavor-related components, 13 amino acids, and 13 other organic compounds) and quantified by ^1H NMR. The data were normalized by Normal Scaling method and only the group means were shown. The numbers in parentheses after each metabolite name indicate the minimum and maximum concentrations in mM. (b) Concentration of kimchi's flavor-related metabolites (mannitol, lactate, ethanol, and acetate) in each pretreatment group. no PEF S-10: No treatment of PEF then brining with 10 g/L NaCl solution, PEF-S05: sample pretreated by PEF and brining using 5 g/L NaCl solution, PEF10: sample pretreated by PEF and brining using 10 g/L NaCl solution, PEF-S15: sample pretreated by PEF and brining using 15 g/L NaCl solution. Error bars depict the standard error of the mean ($n = 3/\text{group}$).

lower M values indicate that the growth rate of TLAB was delayed. R^2_{adj} , which is closer to 1 with lower values of RMSE, indicates that the sum of squares showed a good fit for the kinetic models (McMeekin et al., 2002). Based on the results for this parameter, both models showed acceptable performance in predicting the growth of TLAB in Chinese cabbage kimchi. Similar results showed that the Logistic and Gompertz models were the best-fit models for describing the growth behavior of mustard leaf kimchi (Lee et al., 2021). For the PEF-group, the value of B was 0.09–0.15 and M was increased to 7.750; at the higher NaCl concentration applications, the B-value decreased, and the M-value increased more than the no PEF-S10.

The TLAB results are shown in Fig. 5d. The TLAB count of all groups were similar on day 1 and ranged between 6.34 and 6.47 log CFU/g. After day 7, the no PEF-S10 (7.28 log CFU/g) showed a higher TLAB count than the PEF-group (6.80–6.98 log CFU/g) and this trend was maintained up to day 28. Over day 21, the TLAB of the no PEF-S10 group decreased, whereas that of the PEF group increased slightly. This result was similar to that of another study, which showed that PEF pretreatment (1.7–2.5 kV/cm) on red cherries decreased the LAB growth rate, although the mechanism was unclear (Sotelo et al., 2018). Furthermore, the electroporation on LAB (*L. plantarum* 564) decreased the rate of early log growth phase; however, accelerated the rate of late log phase which indicated slow LAB growth rate (Seratlić et al., 2013). However, many studies have confirmed that pretreatment of PEF with low energy enhances microbial activity and increases the production of metabolites from LAB; thus, the various factors that affect PEF conditions should be further investigated in the absence of PEF-S10 and the growth rate and

quantity of LAB metabolites (Kanafusa et al., 2021). Kimchi is a representative food group fermented by LAB, which provides unique flavors and sensory properties (Lee et al., 2021). However, the continuous growth of LAB, which originate from raw materials such as Chinese cabbage and garlic, induces excessive acidification (sourness) and over-softening of the texture, which can decrease the quality of kimchi (Lee et al., 2021).

Regarding these results, PEF treatment could help to decrease the LAB growth rate due to the cellular structure maintenance, which is particularly relevant in the slow fermentation rates of kimchi.

3.5. General fermentation quality characteristics and sensory properties

The results of pH, total acidity (TA), salinity, and sensory evaluations are illustrated in Fig. 5. PEF pretreatment resulted in a higher pH and lower TA than did no PEF-S10 during fermentation, although the initial TA (days 1–7) showed no significant difference between the groups (Fig. 5a–c). The initial TA ranged between 0.18 and 0.20; however, the PEF-treated group showed a lower TA (0.40 ± 0.01 to 0.52 ± 0.01) than did the no PEF-S10 (0.70 ± 0.01) on day 15. At the end of fermentation, the no PEF-S10 exhibited the highest TA (0.73 ± 0.01) among the groups. Salinity was higher in the no PEF-S10 (1.86 ± 0.03 %) than in the PEF-treated group (1.55 ± 0.03 – 1.68 ± 0.01 %); however, after a day, the salinity showed little difference between the groups, ranging between approximately 1.5 and 1.8 % (Fig. 5c). As LAB growth was delayed in the PEF-treated group, sensory properties of kimchi were influenced; the green taste and smell, indicating early fermentation

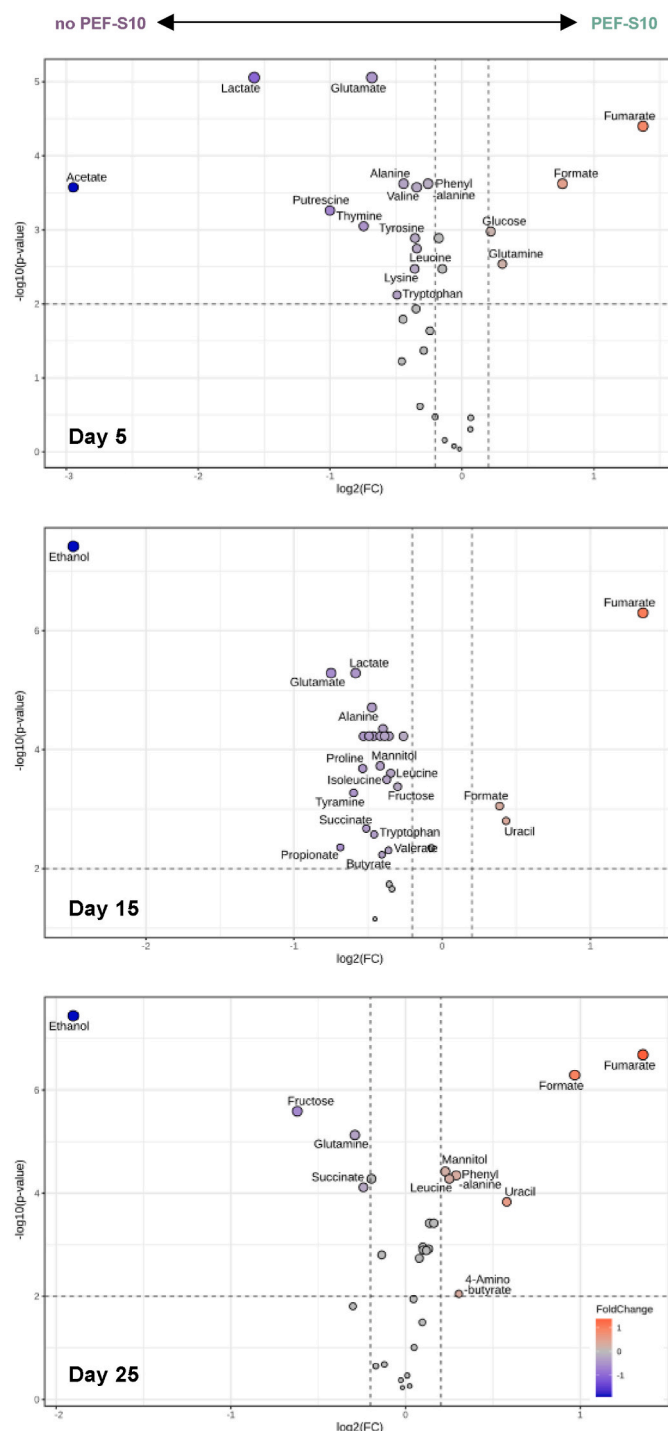


Fig. 4. Volcano plots of the most significant metabolite changes between no PEF-S10 and PEF-S10 groups during fermentation (5, 15, and 25 days). The top left (blue color tone) and top right (red color tone) metabolites indicate the compounds that were significantly increased in no PEF-S10 and PEF-S10 groups, respectively (fold change threshold, 1.15; adjusted p-value threshold, 0.01). no PEF S-10: No treatment of PEF then brining with 10 g/L NaCl solution, PEF-S05: sample pretreated by PEF and brining using 5 g/L NaCl solution, PEF10: sample pretreated by PEF and brining using 10 g/L NaCl solution, PEF-S15: sample pretreated by PEF and brining using 15 g/L NaCl solution.

properties, were the highest in PEF-S15, and the PEF-treated group showed a greater difference compared to the No PEF-S10. Conversely, the acidic smell and taste were the highest in no PEF-S10, indicating that higher LAB growth levels generated lactic acid during the fermentation

process.

The pH of kimchi typically ranged above 5.0 during the initial stage (days 1–5), between 4.2 and 5.0 during the optimal fermentation period (days 5–10), and dropped below 4.0 in the over-ripening stage (days 20–30) according to (Alimentarius, 2001). Similarly, the acidity levels were below 0.2 in the initial stage, between 0.4 and 0.9 during optimal fermentation, and rose above 0.9 in the over-ripening stage when stored at 6 °C, as noted by (Jung et al., 2014). In line with our findings, kimchi made with radishes pretreated with PEF and brined with 10 g/L NaCl solution exhibited a higher pH, except on day 1, and maintained lower TA throughout the fermentation process. Salt is a critical factor in kimchi production that influences texture, LAB composition, growth rate, and flavor (Chang et al., 2011). The optimal NaCl concentration ranged between 1.5 and 3.0 %. As the concentration of NaCl decreased, the fermentation rate increased, thereby enhancing acidity and softening of texture relative to higher concentrations of NaCl (Hong et al., 2021). Kim et al. (2024) reported that radishes brined after PEF pretreatment exhibited a low salinity owing to the increased moisture content, which expanded the cell volume with water and NaCl.

Kimchi is initially fermented by aerobic bacteria and subsequently by anaerobic bacteria during the optimal fermentation period, resulting in a product rich in LAB at approximately 10^7 – 10^8 CFU/g. Throughout the stages of kimchi fermentation—initial, optimal, and over-ripening—the LAB content ranged between 7.0 and 7.5 log CFU/mL, 8.0 and 8.5 log CFU/mL, and below 7.5 log CFU/mL, respectively. The yeast and mold contents at these stages were 3.24, 3.33, and 3.75, respectively (Jung et al., 2014). Kim et al. (2024) reported that PEF pretreatment hindered LAB growth during the early stages of radish kimchi fermentation. This effect was attributed to the preservation of cellular structure and an increase in the release of reducing sugars through pores created in the cell membrane by PEF during the brining process. Consequently, the fresh taste associated with the green smell and taste persisted longer than that of no PEF-S10. Regarding general fermentation quality characteristics, PEF pretreatment resulted in a higher pH and lower TA throughout fermentation, along with a reduced LAB growth rate, which prolonged the freshness of the taste compared with the no PEF-S10.

4. Conclusion

This study demonstrated that pretreatment with PEF could maintain the cellular structure and slow LAB growth in Chinese cabbage kimchi, owing to homogeneous NaCl distribution. Moreover, PEF application dramatically reduced the amount of water required to dilute the NaCl brining solution into waste. Specifically, as the NaCl concentration in the brining solution decreased, the growth behavior and quality properties of the sample became similar to those of the sample without PEF-S10. TLAB was lower in the PEF group during storage than in the no PEF-S10 group, indicating that pretreatment with PEF increased the shelf life of the sample compared to the untreated sample. Additionally, the Logistic and Gompertz models were both acceptable for predicting the growth, and both models confirmed that the PEF group had a lower specific growth rate than the no PEF-S10. Furthermore, kimchi showed no significant difference in fermentation quality, even when a low NaCl concentration of the brining solution was applied to the PEF-treated group. Although kimchi produced using PEF technology exhibits differences in the microbial community of LAB and sensory properties, PEF technology effectively extends storage periods and reduces NaCl concentration in the brining solution. These findings provide a scientific foundation for the application of PEF in kimchi production. In addition, this study presents opportunities for further exploration. The effectiveness of PEF may vary depending on the type of kimchi, raw materials, and processing conditions. Therefore, optimization of field-scale parameters is crucial to ensure reproducibility and consistent product quality. Moreover, integrating microbial community analysis with metagenomics and fungus-specific primer techniques will enhance the understanding of fermentation control mechanisms under PEF

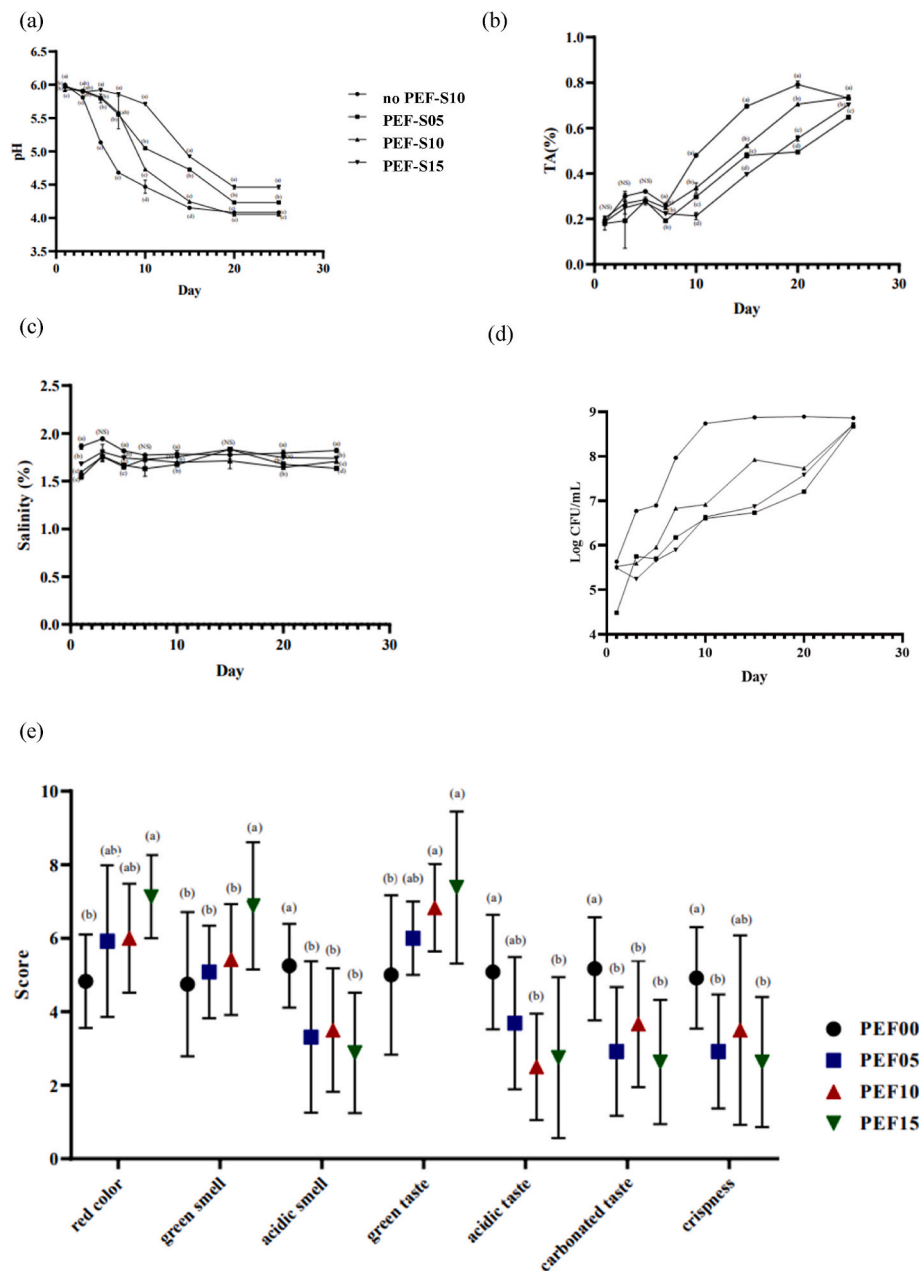


Fig. 5. General quality characteristics and sensory evaluation (multiple comparison test) of kimchi pretreated by Pulsed electric fields (PEF). no PEF S-10: No treatment of PEF then brining with 10 g/L NaCl solution, PEF-S05: sample pretreated by PEF and brining using 5 g/L NaCl solution, PEF10: sample pretreated by PEF and brining using 10 g/L NaCl solution, PEF-S15: sample pretreated by PEF and brining using 15 g/L NaCl solution. (a): pH, (b): TA, (c): salinity, and (d): TLAB and (e): sensory evaluation. Error bars depict the standard error of the mean and the mean values with different letters are significantly different ($p < 0.05$).

treatment. Expanding this approach to other fermented vegetables could accelerate the practical adoption of PEF technology in the global fermented food industry. Future studies focusing on process scalability, energy efficiency, and sensory optimization will further clarify the technological feasibility and commercial potential of PEF-assisted kimchi production.

CRediT authorship contribution statement

Si-Yeon Kim: Writing – original draft, Investigation, Data curation, Conceptualization. **Se-Ho Jeong:** Writing – review & editing, Validation, Data curation. **Yang-An Kang:** Software, Data curation. **Jiyeun Jung:** Data curation. **Ki-Min Lee:** Data curation. **Emmanuel Hitayezu:** Writing – review & editing, Formal analysis. **Intan Rizki Mauliasari:**

Writing – review & editing, Data curation. **Kwang Hyun Cha:** Writing – review & editing, Investigation. **Dong-Un Lee:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Funding

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET), funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA), through the Agricultural Microbiome R&D Program for Advancing Innovative Technology (RS-2024-00396964) and the High Value-Added Food Technology Development Program (grant number 322018).

Table 1
Parameters of predictive 4 models for TLAB of Kimchi pretreated by PEF with different salt concentrations of the brining solution during fermentation at 4 °C.

Sample	Model	Best-fit values			Goodness of fit				
		$y_0^{(2)}$ (log CFU/g)	y_{max} (log CFU/g)	B	M (d)	RMSE	R^2_{adj}	R^2	Sum of Sq
no PEF S10 ⁽¹⁾	Logistic	5.515	8.537	0.176	5.332	0.093	0.9521	0.988	0.034
	Gompertz	6.112	8.558	0.150	5.230	0.095	0.9495	0.987	0.036
PEF-S05	Logistic	5.508	8.125	0.146	5.354	0.020	0.9966	0.999	0.002
	Gompertz	6.055	8.151	0.130	5.480	0.022	0.9958	0.999	0.002
PEF-S10	Logistic	5.177	8.359	0.083	6.614	0.001	1	1	0.000
	Gompertz	5.8	8.452	0.070	6.750	0.002	0.999	1	0.000
PEF-S15	Logistic	5.454	8.283	0.114	7.750	0.057	0.9724	0.993	0.013
	Gompertz	5.849	8.398	0.090	6.650	0.059	0.9705	0.993	0.014

⁽¹⁾ no PEF S10: No treatment of PEF then brining with 10 g/L NaCl solution, PEF-S05: sample pretreated by PEF and brining using 5 g/L NaCl solution, PEF10: sample pretreated by PEF and brining using 10 g/L NaCl solution, PEF-S15: sample pretreated by PEF and brining using 15 g/L NaCl solution.

⁽²⁾ $y(t)$ is the microbial count at the time, t , y_0 is the microbial count at the initial time (day), M is the time at which the absolute growth rate is maximum, and B is the maximum relative growth rate at t . Regression coefficient (R^2), the adjusted coefficient of determination (R^2_{adj}), root mean square error (RMSE), and sum of square (Sum Sq).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This research was supported by the Chung-Ang University Graduate Research Scholarship in 2024.

Data availability

No data was used for the research described in the article.

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